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Strong larvicidal properties of *Argemone mexicana* L. against medically important vectors *Culex pipiens* and *Aedes aegypti*

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Abstract
Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, however, no part of the world is free from vector-borne diseases. One of the most effective alternative approaches under the environmental sustainable control program is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple method of mosquito control. The current study was undertaken to investigate the larvicidal activity of phytochemicals from *A. Mexicana* Linn. Against the medically important vectors *Cx. pipiens* and *Ae. Aegypti.* Leaf water extract as well as different column chromatography elutes (hexane, chloroform, methanol and water) extracted from the seeds of *A. mexicana* were bio assayed against 3rd larval instars of *Cx. pipiens* and *Ae. Aegypti.* The observations recorded after 24 hour and 48 hour of treatment showed a strong significant larvicidal activity of *A. mexicana* seeds chloroform extract followed by seeds methanol extract against 3rd instar larvae of *Cx. pipiens* with LC_{50} values 9.63, and 23.29 mg/l, respectively. In comparison, *Ae. Aegypti* showed a slightly insignificant difference in response towards treatment with chloroform and methanol seed extracts with LC_{50} values 12.59 and 25.21 mg/l, respectively. In general, *A. mexicana* plant extracts have potential for the development of new and safe control products for mosquitoes. Complementary studies on isolation of bioactive ingredients may provide innovative lead products for efficient and effective field application of mosquito control.

Keywords: larvicidal, *Argemone mexicana,* mosquito, *Culex pipiens* and *Aedes Aegypti.*

1. Introduction

Mosquitos are very important medical vector for diseases. They affect the socioeconomic status of many countries and they are important pest against human causing allergy and annoyance too Govindarajan M *et al.* [1]. *Culex* species are important vectors of human pathogens world-wide including the aetiologic agents of different forms of encephalitis, Rift valley fever, and lymphatic filariases. The most important of the *Culex* vectors are members of the *Cx. pipiens* complex. More than 100 million people are infected worldwide with the *Wuchereria bancrofti* form of lymphatic filariasis that is transmitted primarily by *Cx. pipiens* complex in urban and suburban areas, and around 43 million filariasis cases are extremely disabled UNDP/World. [2]. *Aedes Aegypti,* another genus of dangerous mosquitoes and has a worldwide distribution, where it is the main vector of both dengue and yellow fever viruses Sinkins S [3]. During the Spanish-American War, US troops suffered more casualties from yellow fever transmitted by *Ae. Aegypti* than from enemy fire Tabachnick WJ [4]. In general, mosquitoes have strong resistance response toward the conventional chemicals that may be applied for its control leading to potential threat to the global public health Karunamoorthi K *et al.* [5]. Consequently, this important factor, and other numerous drawbacks WHO [6] of synthetic insecticide, have demanded the search for effective and safe biodegradable control agents. According to Shaalan *et al.* [7], plant phytochemicals of potential insecticidal activity can play an important role in the breakdown of the transmission of mosquito-borne diseases.
Wild flora that characterized with its tolerance of drought, poor soil, and often being the only cover on new road cuttings or verges reveal much high concentrations of natural bioactive chemicals Selmar D *et al.* [8]. The bioactive chemicals from these type of easily available plants in semi-arid countries represents alternative sources of natural chemicals that may play roles as medicines or biodegradable pesticides at affordable prices to the market Isman MB *et al* [9].

Floral natural chemicals are basically secondary metabolites that serve as means of the plants defense mechanism to resist the continuous selection pressure from herbivore predators and other environmental factors. Several phytochemicals that were classified to natural chemicals groups such as alkaloids, terpenoids, steroids, phenolics and essential oils from different plants have been stated previously for their insecticidal activities Kaur Mann a *et al.* [10]. From previous studies it was noticed that insecticidal effects of botanical extracts vary not only according to plant species, parts used, insect species and geographical varities, but also due to extraction methodology adopted and the polarity of the solvents used during extraction process Ghosh A *et al.* [11].

Argemone mexicana (Fam: Papaveraceae) is an exotic weed indigenous in South America but has widespread distribution in many tropical and sub-tropical countries including West Africa Ibrahim HA *et al.* [12]. This plant is common everywhere by roadsides and fields and causes agricultural problems as an injurious weed Bhalke RD *et al* [13]. The plant phytochemicals extracted from leaves and seeds are reported to find application in maintaining normal blood circulation and cholesterol level in human body Albuquerque U *et al.* [14]; these plant parts possess anti-venom property as well Minu V and Brahmachari G *et al.* [15, 16].

The aim of the current work is to investigate the larvicidal activity of phytochemicals extracted from different parts of *Argemone mexicana* Linn. Against the medically important vectors *Cx. pipiens* and *Ae. Aegypti.*

2. Materials and Methods

2.1 Maintenance of mosquito colonies

Adult mosquitoes were sustained in wired cages, at 27 ± 2 °C and 75–85% RH and fed on sterile 10% sucrose solution. Yeast suspension (5%) was used as food source for larvae. Pupae were rinsed and transferred to sterile distilled water and placed in separate cages until adult emergence. Production of eggs was convinced after providing blood meal (rabbit) to the females. Egg were transferred to plastic containers filled with dechlorinated water for larvae mass production. *Cx. Pipiens* and *Ae. Aegypti* colonies were separated from each other by labeling the cages and containers.

2.2 plant material

Mexican poppy *Argemone mexicana* L. (Fam: Papaveraceae) were collected by the author from the lemon fields canals of Guadalajara, Mexico. At the time of collection the plant was during its flowering phase (May/2011). The plant was authenticated at the herbarium of Botany Department, Faculty of Science, Mansoura University. Plant parts under investigation were leaves and seeds.

2.2.1 Preparation of extracts

Collected leaves were washed thoroughly under running tap water. It was dried on tissue paper for three days at room temperature. Further it was crushed properly using electric grinder and 200 ml of distilled water was added to the leaves powder to make water extract by shaking for 48 hours using electric shaker. Filtration and centrifugation of the extract yielded a phytochemicals residue which was dried in vacuum oven at 40 °C for full night. Stock solution was prepared by dissolving 1 g of dried residue in 10 ml of distilled water and used for the bioassay study.

Seeds were collected from the plant and washed with distilled water then dried on canson paper in laboratory at 37±1 ^{0}C for two days. The dried seeds were powdered in a grinding machine and 100 gm powdered material was extracted with methanol for 3-4 days by soaking technique. The extract was filtered using filter paper (Whatman No. 1) and the filtrate was subjected to the rotary vacuum evaporator.

The methanol extract, a semi-solid gummy yellow brownish bulk, was passed through column chromatograph eluted with different polarity solvents such as hexane, chloroform, methanol and water. These eluates (extracts) obtained were stored in a refrigerator at 4 ^{0}C for further experimentation.

2.3 larvicidal bioassay test

The larval susceptibility tests were carried according to standard procedures World Health Organization [17]. Different concentrations of extracts were prepared and larvae of *Cx. pipiens* and *Ae. Aegypti* were placed in each test solution to observe the larvicidal property as per the following procedure. Groups of 25 larvae were placed in glass beakers containing 200 ml of the plant extract solution. The larval mortality of the 3rd instar was observed after using different concentrations (5, 20, 40, 60, 80 and 100 mg/l) from the tested extracts. Control experiments with solvent only (+ve control) and without solvent (-ve control) were run in parallel. The larvae in each solution were then left for 24 h, and 48 h the number of dead larvae were counted after 24 h and 48 h of exposure, and the percentage mortality was reported from the average of four replicates. Mortality was recorded and when a control mortality ranged from 5 – 20 per cent, it was corrected by Abbott's Abbott WS [18] formula.

3. Results and discussion

Data was analyzed by Ldp-line computerized program to determine the dose-response relationship by probit analysis [19]. Based on Abbott's formula Abbott WS [18], mortality data was corrected.

Larvicidal tests with different solvent extracts produced different results because the solvents polarity play important role in fractionating the chemical components of the plant crude extract. After 24 hours of treatment, the data results showed strong significant larvicidal activity of *A. mexicana* seeds chloroform extract followed by seeds methanol extract against 3rd instar larvae of *Cx. pipiens* with LC_{50} values 9.63, and 23.29 mg/l, respectively. In comparison, *Ae. Aegypti* showed LC_{50} values 12.59 and 25.21 mg/l with chloroform and methanol seed extracts, respectively (Table 1 & Figure 1). While, increasing the time of exposure to 48 hour led to increase the relative toxicity against *Cx. pipiens* and *Ae. Aegypti* in all treatments as showed in Table 1 and figure 2. A previous study done by Warikoo & Kumar [20] using leaf stem and root extracts of deleterious weed *A. mexicana* prepared in five different solvents (petroleum ether, hexane, benzene, acetone, and ethanol), against *Ae. Aegypti* larvae revealed 1.6- to 2.4-fold higher larvicidal efficacy of root extracts than those prepared from the leaves and stem of *A. mexicana*. The hexane root extract of *A. mexicana* was found to be the most effective larvicide with LC_{50} value of 91.331 ppm after 24 h of exposure causing 1.8 and 2.4 fold more toxicity as compared to the hexane leaf and stem extracts, respectively. While *Anopheles* larvae showed a significant toxicity tolerance with LC_{50} value 161.1 ppm Elawad LM *et al.* [21] after the same exposure time.

Additionally, the results recorded by Sakthivadivel *et al.* [22] indicated that among the selected plants for the study, *A. mexicana* showed the maximum larvicidal activity with an LC_{50} value of 48.89 ppm. Its toxicity was enhanced when the extract was mixed (1:1) with that of *C. dentata* as the LC_{50} value became 28.60 ppm indicating synergistic action of *A. mexicana*. The chloroform extract of *Plumbago zeylanica*, *P. dawei* and *P. stenophylla* roots have shown high larvicidal activities against *An. gambiae* (4.1, 6.4 and 6.7 mg/ml respectively) Maniafu BM *et al.* [23] but non-significant susceptibility of *Cx. pipiens* was documented by Manimaran, *et al.* [24].

The larvicidal activity of the different extracts of *A. mexicana* against *Cx. pipiens* in the present study is supported by the findings of Saktivadivel & Daniel [25], who reported the LC_{50} values as 30.47 and 24.17 and LC90 as 246.33 and 184.99 ppm for leaves and seeds respectively. Karmegan *et al.* [26] tested the leaf extract of *A. mexicana* against *Cx. quinquefasciatus.* The results showed potential larvicidal activity causing 100% mortality at 250 ppm of all tested extracts.

Table 1: Larvicidal efficacy of different extracts from *A. mexicana* against *Cx. pipiens* and *Ae. Aegypti* mosquito larvae after 24 and 48 hour of treatment.

Tested Insect larvae	Treatment Extract of *A. mexicana*	24 hour exposure time			48 hour exposure time		
		LC_{50}* (mg/l)	LC_{90}* (mg/l)	X^2	LC_{50}* (mg/l)	LC_{90}* (mg/l)	X^2
Cx. pipiens	H_2O leaf extract	77.82	281.76	0.17	61.78	174.23	0.15
	hexane seed extract	56.15	296.59	0.15	44.83	177.65	0.17
	$CHCl_3$ seed extract	9.63*	23.21*	0.16	5.42	13.84*	0.16
	MeOH seed extract	23.29*	69.48*	0.17	16.18	48.37*	0.16
	H_2O seed extract	88.47	296.72	0.17	70.48	236.82	0.17
Ae. Aegypti	H_2O leaf extract	97.65	364.82	0.16	63.48	191.69	0.16
	hexane seed extract	68.57	183.6	0.15	51.38	184.89	0.15
	$CHCl_3$ seed extract	12.59*	30.32*	0.17	8.47	20.63*	0.17
	MeOH seed extract	25.21*	70.48*	0.17	19.58	54.63*	0.17
	H_2O seed extract	89.26	213.6	0.16	81.46	251.18	0.17

***** Significant LC values compared to the highest LC value.

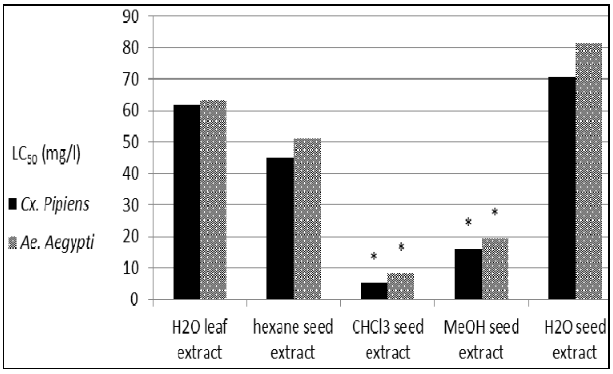

Fig 1: Histograms showing the Relative toxicity (LC_{50}) of different extracts from *A. mexicana* against *Cx. pipiens* and *Ae. Aegypti* mosquito larvae after 24 hour of exposure time.

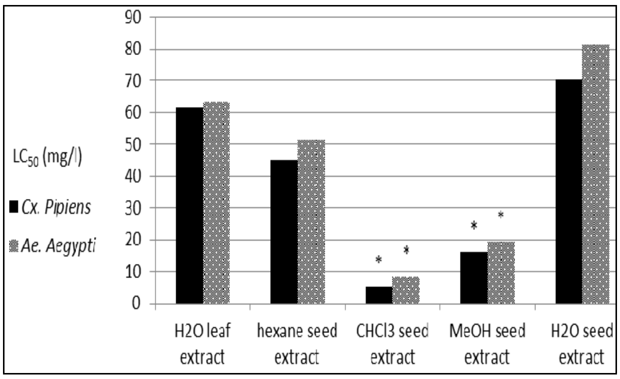

Fig 2: Histograms showing the Relative toxicity (LC_{50}) of different extracts from *A. mexicana* against *Cx. pipiens* and *Ae. Aegypti* mosquito larvae after 48 hour of exposure time.

4. Conclusion

The present study shows that the *Argemone mexicana* plant extracts have potential for the development of new and may be safe control products for mosquitoes. As naturally occurring insecticides, these plant-derived materials could be useful as an alternative for synthetic insecticides. The screening results suggest that the chloroform and methanol extracts of *A.* mexicana seeds are promising in controlling the most important mosquito-borne diseases vectors *Cx. pipiens* and *Ae. Aegypti*. The results also showed a non-significant susceptibility of *Cx. pipiens* in comparison with the larval susceptibility of *Ae. Aegypti* towards the tested extracts. Further studies on isolation of bioactive fraction/constituent may provide futuristic lead products for efficient and effective field application of mosquito control.

5. References

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